MICROSCOPY.[1]

Methylen Blue.—A few points observed in the use of Erlich's methylen blue method by the investigators in the Marine Biological Laboratory at Woods Holl, Mass. may be of general interest.

This method has been successfully applied during the past summer to the study of the nervous system in a great variety of forms including vertebrates, crustacea, annelids, echinoderms and tunicates.

Ehrlich's *intra vitam* methylen blue prepared by Grübler was used for staining the nerve tissues. The stain was applied by injecting a 1–½ per cent solution of the methylen blue made in normal salt solution, into the blood vessels, body cavity or lymph spaces or by immersing small animals or excised pieces of nerve tissue in a weak solution.

The method of application and strength of the solution were determined by experiment for each animal and tissue. During the action of the stain, the animal or tissue was kept as nearly as possible in its normal condition. Everything seems to depend on keeping the tissue alive, and in bringing the stain in contact with it in a solution of a strength suitable for obtaining the best results.

The abundant supply of oxygen to the staining tissue was of great importance in some cases, while in others it seemed to make little difference.

It was found, as suggested by Dr. C. Huber, that animals which live in the dark, stain better in the dark than in the light.

The relaxation of the tissues by the use of chloroform or chloral hydrate seemed to be more favorable for the staining of some elements of the nervous system, while others did not stain which stained in the unchloroformed animal.

It was found that recently caught and perfectly normal animals stained more satisfactorily than those which had been kept in confinement for some time, unless under very favorable conditions.

In the case of the dogfish, active animals were killed by decapitation. The stain was applied by injecting a 1–½ per cent solution of the methylen blue into the blood vessels for the central nervous system and by immersing small pieces of nerve tissue in a weak solution of the stain for the sense organs.

The length of time required for the *intra vitam* staining varied widely, annelids requiring 4–5 hours, while dogfish only require 1–½ hours, either by injection or by immersing the tissue in the stain.

[1]Edited by C. O. Whitman, University of Chicago.

When small transparent pieces of tissue were to be examined, they were fixed in a saturated solution of picrate of ammonia in distilled water from 2–4 hours and were then mounted in a mixture of equal parts of pure glycerine and distilled water to which a small quantity of picrate of ammonia is added. When opaque or large pieces were fixed in this way they were sectioned by the freezing method. After fixing in the picrate of ammonia, the tissue was placed in a saturated solution of sugar for one hour and was then transferred to a piece of blotting paper to remove the syrup from its surface. It was then placed in a thick solution of gum arabic for fifteen minutes and then transferred to the plate of the freezing microtome where it was frozen by means of liquid carbonic acid. The sections were mounted in dilute glycerine as in the other case. The principal advantage of this method is its rapidity, but neither serial sections nor those of equal thickness can be obtained.

In order to obtain serial sections by the paraffine method, the tissues were fixed in Berthe's Fluid.[2]

For vertebrates:	For invertebrates:
Molybdate of ammonia, 1 gram.	Molybdate of ammonia, 1 gram.
Distilled water, 10 c. c.	Distilled water, 10 c. c.
Hydrochloric acid, 1 drop.	Peroxide of Hydrogen, ½ c. c.
Peroxide of Hydrogen, 1 c. c.	

A different formula is used for tissues of invertebrates as less oxygen is required than for vertebrates. The fixing fluid must be cooled on ice before placing the tissue in it. After remaining in the cold fixing fluid for from 2–4 hours the tissue is thoroughly washed with cold water which generally takes about two hours although it has been continued for twelve hours without injury.

It is necessary to remove all the molybdate of ammonia by thorough washing if permanent preparations are to be secured.

The tissue is then passed rapidly, ten to fifteen minutes in each, through the ordinary grades of alcohol to absolute, all being kept cold with ice. The tissue should be left in the absolute alcohol for about two hours at a freezing temperature and the alcohol be changed several times. The stain is dissolved by dilute alcohol at ordinary temperatures.

Dr. Huber's plan of placing the tissue directly in cold absolute alcohol on removing it from the water and changing several times for a period of two hours, gave good results.

[2] Archiv. f. Mikros., Anat. Bd. 44, Heft 4.

After thorough dehydration the tissue is placed in xylol for 12–24 hours and changed several times. It is then imbedded in paraffine in the usual way.

The most complete and in every way satisfactory staining of the sensory nervous system was obtained by two or three injections of a ½ per cent solution of Erlich's methylen blue at intervals of from 15 to 20 minutes, both with vertebrates and invertebrates, as suggested by Semi Meyer.[3]

The tissues relaxed after the first injection so that more fluid was introduced by the second and third injections than by the first.

The use of chloroform was found to be wholly unnecessary by this method. Meyer uses a very strong solution of B. X. methylen blue, 5 per cent to 6 per cent, in water.

The paraffine sections should generally be quite thick (45–60 $M\mu$).—A. D. MORRILL, Hamilton College, Clinton, N. Y.

PROCEEDINGS OF SCIENTIFIC SOCIETIES.

American Association for the Advancement of Sciences (continued from page 779).—Prof. A. S. Packard was elected to represent the Association on the American advisory board to coöperate with the American member of the international commission on rules of nomenclature. The following were elected to represent the Association at the International Congress of Geologists to be held in St. Petersburg in September, 1897: Prof. Jas. Hall, Prof. E. D. Cope, Prof. B. K. Emerson, Prof. C. D. Walcott, Prof. W. N. Rice.

The following sectional officers were elected to serve at the meeting of 1897, at Detroit.

Vice-Presidents.—A, Mathematics and Astronomy.—W. W. Beman, of Ann Arbor, Mich. B, Physics.—Carl Barus, of Providence, R. I. C, Chemistry.—W. P. Mason, of Troy, N. Y. D, Mechanical Science and Engineering.—John Galbraith, of Toronto, Can. E, Geology and Geography.—I. C. White, of Morgantown, W. Va. F, Zoology.—G. Brown Goode, of Washington, D. C. G, Botany.—George F. Atkinson, of Ithaca, N. Y. H, Anthropology.—W. J. McGee, of Washington, D. C. I, Social and Economic Science.—Richard T. Colburn, of Elizabeth, N. J.

[3] Archiv. f. Mikros. Anat. Bd. 46. Heft 2, and Bd. 47. Heft 4.